(n=3, $p<0.05$), relative to control. Importantly, flecainide + mannitol decreased θ_L and θ_T by 17 ± 2 and $9 \pm 2\%$ respectively ($p<0.05$), raising AR_θ to 3.3 ± 0.2 ($p<0.05$) relative to mannitol alone. $BaCl_2$ + mannitol increased θ_T (by $15 \pm 3\%$, $p<0.05$) but not θ_L relative to mannitol alone, thereby lowering AR_θ to 2.6 ± 0.1 ($p<0.05$). Interestingly, the computer model of edema and ephaptic coupling recapitulated experimental findings where reducing INa decreased θ and increased AR_θ while reducing IK1 increased θ and decreased AR_θ.

Conclusions: INa and IK1 blockade isotropically affected θ by themselves, but preferentially altered θ_T during edema. This is the first demonstration that altering ionic currents can alter AR_θ, and the mechanism may involve ephaptic coupling.

178-Plat

Myofibroblast-Myocyte Coupling Through Adherens Junctions Slows Cardiac Electrical Propagation

Susan Thompson, Leslie Tung.
Johns Hopkins University, Baltimore, MD, USA.

Following cardiac injury, activated cardiac myofibroblasts can influence tissue electrophysiology. A popular hypothesis for myofibroblast-induced conduction abnormalities is that myofibroblasts can electrically couple to myocytes, thereby providing an additional load. Although the existence of functional electrical coupling is controversial, the commonly observed close proximity of myofibroblast and myocyte membranes suggests that heterocellular communication could be operating. Based on our preliminary results, we tested the hypothesis that cardiac tissue electrophysiology is affected by mechanical coupling through adherens junctions between myofibroblasts and myocytes. Our recently published data show that after application of the excitation-contraction uncoupler, blebbistatin, or the mechanosensitive channel (MSC) blocker, gadolinium or streptomycin, conduction velocity (CV) was dramatically increased to near control levels in fibrotic monolayers (co-cultured myofibroblasts and neonatal rat ventricular myocytes (NRVMs) treated with transforming growth factor-beta). Using pan-cadherin and N-cadherin antibodies, we also visualized cadherin junctions between myocytes and myofibroblasts. We hypothesized that OB-cadherin is the predominant myofibroblast cadherin in the heterocellular junctions because it is upregulated upon fibroblast activation to myofibroblast and is associated with wound healing. However, CV remained slow upon co-culturing OB-cadherin silenced myofibroblasts with cardiomyocytes, and could still be restored with the addition of MSC and contraction blockers, suggesting that OB-cadherin is not the predominant myofibroblast cadherin in heterocellular junctions. Alternatively, longitudinal and transverse CV in NRVM monolayers supplemented with myofibroblasts and neutralizing N-cadherin antibodies was significantly faster ($19. \pm 0.8$ cm/s and 6.6 ± 0.3 cm/s, respectively, n=3) than in myofibroblast-supplemented NRVM monolayers lacking such antibodies (10.4 ± 0.9 cm/s and 4.3 ± 0.5cm/s, respectively, n=3, $P<0.05$). These observations suggest that cardiac conduction can be impaired as a result of coupling of myofibroblasts to myocytes through N-cadherin, which may allow the transmission of tugging forces from the myofibroblasts to activate MSCs.

Platform: Voltage-sensitive Proteins

179-Plat

The Resting and Activated Conformations of the Voltage Sensor of Ci-VSP from Functional and Solvent Accessibility Determinations

Qufei Li[1], Sherry Wanderling[1], Carlos A. Villalba-Galea[2], Eduardo Perozo[1].
[1]Institute for Biophysical Dynamics and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.

The voltage sensor domain (VSD) is responsible for electromechanical transduction in voltage-gated ion channels and enzymes. In all known VSDs, both architecture and voltage-sensing mechanism are conserved: the positive charged residues (R/K) on the fourth transmembrane segment S4 respond to the voltage change across the membrane, which trigger its own conformation change leading to the response of downstream domain. A wealth of biophysical information on voltage sensors in the last two decades has revealed one of the major functional states - ''up'' or activated state. However, the structure and functional properties of the ''down'' or resting state remains controversial. Here, we show electrophysiological and structural studies of the voltage sensor from *Ciona intestinalis* voltage sensitive phosphatase (Ci-VSP), that point to conformational transitions between the resting and activated conformations of the sensor. The voltage dependence of Ci-VSP mutants, analyzed by gating charge measurement in oocytes, show significant shift in their Q-V relationships along the voltage axis (R217E −60 mV, R217Q −20 mV, WT +60 mV, D136N +130 mV). At 0 mV, these mutants populate different functional states under biochemical conditions: WT and D136N mostly in the ''down'' state while R217E is mostly in the ''up'' state. A Ci-VSD biochemical preparation was developed for each of the four mutants and studied by site-directed spin labeling EPR (SDSL-EPR) methods in proteoliposomes. Mobility and accessibility information revealed the secondary structure of transmembrane segments and their positions relative to membrane and each other, suggesting the extend and direction of the motion of S4 between ''up'' and ''down'' states. These results are consistent with the down movement of S4 under hyperpolarization and render critical structural information, that allow us to propose a gating mechanism for Ci-VSD.

180-Plat

Control of Hv1 Voltage-Gated Proton Channel Opening by Changes in the Transmembrane Voltage and pH Gradients

Carlos A. Villalba-Galea, I. Scott Ramsey.
Virginia Commonwealth University School of Medicine, Richmond, VA, USA.

Hv1 proton channels contain a voltage sensor (VS) domain that is homologous to that of tetrameric voltage-gated cation channels (VGCs) and voltage-sensitive phosphatases (VSPs), but cooperative channel gating by changes in both membrane potential (V_m) and the transmembrane pH gradient ($\Delta pH = pH_O - pH_I$) is apparently unique to Hv1. The mechanism underlying V_m-ΔpH coupling in Hv1, which results in a linear −40 mV/ΔpH unit shift in the G_{H+}-V relation, is not well understood. In order to isolate ΔpH-dependent steps in the Hv1 activation pathway, we measured the effect of changing ΔpH on expressed H^+ currents using patch clamp electrophysiology. We show here that Hv1 opening exhibits both voltage-dependent and voltage-independent kinetic components, but closing is purely voltage-dependent. The forward time constant for the final charge-translocating step in Hv1 activation gating is steeply ΔpH-dependent at acidic pH_I but only weakly sensitive to ΔpH at alkaline pH_I. In addition, we find that at high pH_I, a voltage-independent transition becomes rate-limiting for channel opening. Analysis of the relative contributions of voltage-dependent and -independent Hv1 gating components demonstrates that both are necessary to explain the linear V_m-ΔpH relation between pH_I 4.5 and 7.5. The effect of changes in ΔpH on Hv1 gating contrasts with Ci-VSP, indicating that Hv1 is particularly well tuned to respond to small perturbations in ΔpH over the physiological range ($pH_I \approx pH_O$). We propose a new multi-state model to account for complex gating in Hv1 in which VS activation and channel opening occur in distinct transitions and display different sensitivities to changes in ΔpH. V_m-ΔpH coupling in Hv1 thus represents an integrated response to changes in both voltage-dependent and -independent gating steps.

181-Plat

Internal Switch Gates Phosphatase Activity of VSP

Lijun Liu[1], **Susy C. Kohout**[2], Qiang Xu[1], Simone Mueller[1], Ehud Y. Isacoff[2], Dan Minor[1].
[1]University of California, San Francisco, San Francisco, CA, USA, [2]University of California, Berkeley, Berkeley, CA, USA.

Voltage regulation is responsible for a host of processes in the cell from muscle contraction to neuronal action potentials. In the Ciona intestinalis voltage-sensing phosphatase (Ci-VSP)1, voltage is responsible for controlling lipid phosphatase activity. How this happens is still not known. To address this, we have solved seven crystal structures of the cytosolic fragment of Ci-VSP. The structures reveal three active site conformations, one where the active site is blocked, one where the active site is open and one where the active site is bound with substrate. These three conformations show that the active site is gated. Activity assays on structure-based mutants show that the gate is involved in catalytic turnover as well as substrate specificity. A residue from the C2 domain that lines the active site in Ci-VSP, but not in the homologous phosphatase PTEN, also contributes to selectivity. A potential interaction between the segment of the protein that gates the active site and the inter-domain linker between the voltage sensing domain and the phosphatase suggests a model for coupling the voltage sensor to activity.